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Effect of free nitrous acid pre-treatment on primary sludge at low exposure times

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Abstract

The present study was undertaken to investigate the effect of different Free Nitrous Acid (FNA) concentrations at low pre-treatment times (PTs) (1, 2 and 5 h) and without pH control with mild agitation on primary sludge (PS) biodegradability and methane production (MP). Increasing PTs resulted in an increase in the solubility of the organic matter (around 25%), but not on cell-mortality (>75% in all the cases with FNA) and neither on methane generation. FNA pre-treatment at low PTs improve MP (around 16% at PT of 1h and 650 mg N-NO_2^- /L). However, a similar improvement was found with mild agitation of PS without FNA at 2 and 5 h. Taking into account the potential costs associated with the FNA pre-treatment, a mild agitation without FNA would be preferred to enhance MP in PS.

Keywords: anaerobic digestion; free-nitrous acid pre-treatment; methane production, primary sludge, sludge biodegradability.

1. Introduction

Waste water treatment is a key process in our society to avoid pollution in our water bodies, as well as provide a source of water that can be directly used for irrigation. This last aspect is becoming more important due to climate change, with currently above 46 % of cultivated areas in the world not receiving enough rain water for their agricultural needs (Yannopoulos et al., 2015; Valipour, 2015). Wastewater treatment is increasingly being applied worldwide, increasing as well sludge production. Sludge management is a serious issue since up to one-half of the costs of operating municipal Waste water treatment plants (WWTPs) is associated with sludge treatment and disposal (Lens, 2004; Peces et al., 2016). Anaerobic digestion (AD) is a sludge treatment process used in many WWTPs to stabilize primary sludge (PS) and waste activated sludge (WAS) from aerobic/anoxic biological treatment processes obtaining methane (CH_4) (Climent et al., 2007; Peces et al., 2016). PS is a result of the capture of suspended solids and organics in the primary treatment process through gravitational sedimentation, typically by a primary clarifier. The WAS is obtained from the secondary clarifier after the secondary treatment process whereby microorganisms are used to consume organic matter and nutrients from the wastewater. The different origin of PS and WAS make them to have different characteristics: WAS has a much higher content of microorganisms and proteins but lower fatty acids content and is less biodegradable having therefore a lower methane production potential than PS (Lens, 2004; Sato et al., 2001; Wilson and Novak, 2009; Zhang et al., 2016). On the other hand, PS has less microorganisms and more fatty acids (Cokgor et al., 2009; Peces et al., 2016; Zhang et al., 2016). In many WWTPs both sludge types are mixed before entering the anaerobic digester and also, in many cases, a pre-treatment of this sludge mixture is done to increase its biodegradability and enhance CH_4 production during the digestion process. Recent studies have demonstrated the efficacy of free nitrous acid (FNA) pre-treatment for WAS (Ganda et al., 2016; Ma et al., 2015; Wang et al., 2013, 2014; Wu et al., 2016). FNA, the protonated species of nitrite, can be produced in the same WWTP by the nitritation process of the anaerobic digestion liquor, making it economical and environmental more attractive than other pre-treatment methods. FNA destroy cells and solubilize the extracellular polymeric substances (EPS) (especially proteins and polysaccharides) present in sludge. This causes the release of intracellular and/or extracellular constituents to the aqueous phase (Carrère et al., 2010; Ma et al., 2015), which are more easily biodegradable during AD, thereby enhancing methane production (Wang et al., 2013).

On the other hand, the only study present in the literature where FNA pre-treatment is used in PS suggests that this pre-treatment compromises the methane production (MP) (Zhang et al. 2016). In that study PS was exposed to different concentrations of FNA (from 0.77 to 3.85 mg N-HNO_2/L) during 24 h. In all tests an increase on the soluble chemical oxygen demand (COD) concentration was detected. However, lower methane production rate and methane production potential was observed in the PS treated with FNA as compared with the non-treated control. Their results indicated that FNA pre-treatment at 24 h resulted in the methane potential reduction of 1–7%.

In another study, Zahedi et al. (2016) applied an FNA pre-treatment to a mixture of PS and WAS obtaining an improvement on the MP when the pre-treatment time was reduced to 5h. If that improvement was linked only to WAS or to PS remained unclear.

In the present study, the effect of the FNA pre-treatment on PS characteristics was assessed at 4 different FNA ranges (0; 1.2-3.9; 1.7-5.6; and 2.6-7.3 mg N-HNO_2/L) and

3 different exposure times (1, 2 and 5 h). Also the effect of mild mixing was studied. To evaluate the effect of the different pre-treatments on the biochemical methane potential (BMP), BMP tests were also conducted in triplicates for each conditions tested.

This is the first study reporting the effect of the FNA pre-treatment in PS to enhance MP at low pre-treatment times and without pH control. Former studies have focused on long pre-treatment times (24h) and addition of acid to have a fixed control of the pH values. These aspects are important, since low pre-treatment times are preferred for real application, reducing the volume of the pretreatment tank needed. Also, no pH control means substantial savings and lower risk from handling acids during the FNA pre-treatment process.

2. Materials and Methods

2.1 Substrate and Inoculum

The substrates that were used in batch tests are PS that withdrawn from the primary sludge clarifier from a WWTP (Lleida WWTP, Catalonia, Spain). The pH, total solids (TS), volatile solids (VS) and soluble chemical oxygen demand (SCOD) concentrations in the PS were 5.2±0.1, 42±1 g TS/kg, 32±1 g VS/kg and 157±2 mg SCOD/g VS, respectively.

For the methanogenic studies (BMP tests), inoculum from the mesophilic anaerobic digester present at the same WWTP was collected. The pH, TS, VS and SCOD concentrations in the inoculum were 7.4±0.1; 23±0 g TS/kg, 14±0 g VS/kg and 29±0 g SCOD/g VS, respectively.

2.2. FNA pre-treatment methodology

Four laboratory-scale continuously stirred polyethylene reactors were used in these studies. The pre-treatment reactors had a working volume of 1L and were mixed at a speed of 100 rpm with four mechanical stirrers (FLUCOMATIC 6 system, SELECTA S.A). The concentrations of nitrite used were the same as those used by Zahedi et al., (2016), corresponding to 0 (Test 1), 350 (Test 2), 500 (Test 3) and 650 mg N-NO_2^-/L (Test 4). The FNA concentration slightly varied throughout each of the tests due to the fact that pH was not controlled and raised from 5.3 to 5.8 in the tests where nitrite was added. The FNA concentration was calculated using the formula $S_{N-NO_2^-} \big/ K_a \times 10^{pH}$ with the Ka value determined using the formula $K_a = e^{-2,300 / (273+T)}$ for a given temperature T (°C) (Anthonisen et al., 1976). Different volumes of a nitrite stock solution (118.3 g $NaNO_2$/L) were supplemented in each reactor at the beginning of the experiment in order to achieve the targeted nitrite concentrations (Table 1). To unravel both the effect of the pre-treatment time and the effect of the FNA concentration in the characteristics of the primary sludge, sludge samples were taken at different exposure times (1, 2 and 5 hours). The pre-treatment assays were carried out at room temperature (~ 25ºC).

2.3. Methane generation studies

To investigate the effect of FNA pre-treatment on the methane production potential of PS, BMP tests were used. The BMP tests were carried out in 250 mL serum bottles (with a working volume of 100 mL). Each BMP test contained 82 mg of inoculum and 18 mg of PS to maintain an inoculum to PS ratio of 2 on a dry VS basis. All the bottles were closed and maintained in a mesophilic temperature controlled (at 37ºC) employing a ST 700 incubator (POL-EKO). To ensure sufficient mixing, the bottles were continuously shacked at 150 rpm using a KS 260 basic orbital shaker (IKA).

To assess the methane generation of inoculums, as well as to evaluate the effect that nitrite could potentially have on the activity of the inoculums four blanks were conducted (Blank I, II, III and IV). Blank I contained inoculum and Milli-Q water without pretreated sludge. Blanks II, III and IV were identical to Blank I but with the addition of nitrite stock solution, which resulted in an initial nitrite concentrations of around 63, 90 and 117 mg N-NO_2^-/L, respectively, mimicking the nitrite concentrations present in the BMP tests when FNA pretreated sludge was added. Methane production (MP, milliliters of methane produced) from the PS was obtained by subtracting the MP from the inoculum (Blank 1) according to the same methodology used in Zahedi et al., (2016). MP and specific MP (SMP, milliliters of methane produced per gram of VS added) have been expressed under normal conditions (P=1 atm and T= 0ºC). The BMP tests were carried out in triplicates and lasted for 45 days.

2.4. Chemical and microbial analyses

TS, VS, soluble Kjeldahl nitrogen (SKN) and SCOD were determined according to standard methods (APHA, 1995). NH_4^+ was analyzed via ion chromatography (ICS5000, DIONEX). Proteins were measured with the Folin Phenol Reagent according to Lowry (1951) and Peterson (1977) and carbohydrates were measured using a colorimetric method (fenol plus sulfhidric acid) according to Dubois et al. (1956). Biogas was monitored on a daily basis during the first 10 days and every 2-4 days afterwards. A pressure sensor PM7097 (IFM electronic) was used to determine the pressure increase in the headspace volume (150 mL) and cumulative gas production was calculated from these pressure increase. An infrared specific CH_4 sensor: GasTech S-Guard (GIR-3000 Model), that was calibrated using a commercial 100% CH_4 bottle (Abelló Linde S.A.), was used to determine of CH_4 in the biogas.

Regarding to microbiological analysis, Live/Dead® BacLight™ bacterial viability kit (Molecule Probes, L-7012) was used to identify viable and dead /damaged cells according to Zahedi et al. (2016). With this stain, cells with damaged cell membrane (assuming to be death) are stained in red while viable cells (with no membrane damage) are appearing in green. Twenty images from each preparation were taken with a Nikon confocal laser-scanning microscope using Plan-Apochromat 63xoil objective for quantification of the viable and non-viable cells.

3. Results and discussion

3.1. FNA and pH variation

A slight variation on the pH occurred during the pre-treatment in those tests where nitrite was added causing a variation on the level of FNA. This was due to the fact that no pH control was applied. Figure 1 shows the pH and FNA changes during the duration of all the tests. pH was measured every 10 s along the 5 hours and FNA was calculated for each pH measurement using the formula described above. pH was relatively constant during the first 45 min of the pre-treatment in all tests except the one exposed to the highest nitrite concentration which slightly decreased. After this, a sharp increase on pH was observed in all tests where nitrite was added till reaching a stable value around 5.8. This caused a decrease on the FNA concentration in all tests corresponding to a decrease of 60, 64 and 67% of the original FNA concentration in Tests 2, 3 and 4 respectively.

The effect of pH changes on PS not exposed to FNA was individually assessed (Fig 1a) and no changes were detected. This indicates that the changes on the pH were due to the effect that nitrite/FNA had on the PS.

3.2. Toxicity of FNA to PS

The biocidal effect of the different concentrations of FNA to PS was assessed after 1, 2 and 5h of pre-treatment. Figure 2 shows the percentages of non viable microorganisms detected after the different exposure times assessed. A biocidal effect of FNA on microbial cells is clearly observed in all cases. The PS contained around 30% of damaged cells. In the control test (test 1) with no nitrite addition, this percentage increased to 44±3 % after 2-5 h of sludge mixing. When FNA was added, the percentage of damaged cells increased to 80 or 90% depending on the concentration of FNA used. Similar levels of mortality were detected by Jiang et al. (2011) when exposing an anaerobic biofilm to similar levels of FNA or in Zahedi et al., (2016) when exposing mixed sludge. This indicates that sludge viability is quickly compromised when FNA is added and low exposure times (1h) are sufficient to damage the majority of the cells present in the PS.

3.3. Effect of FNA pre-treatment on PS solubilization

Figure 3 shows the influence of the FNA concentration on the different organic and nitrogenous compounds of the PS at different pre-treatment times.

In terms of SCOD only a slight increase in the FNA pre-treated test was detected at the highest exposure time as compared to the control test with no addition of nitrite. On the other hand, FNA pre-treatment resulted in an increase of soluble carbohydrates, proteins and SKN. While the first ones increase with FNA concentration and exposure time, the increment in soluble proteins and SKN occurred in a similar manner under all the FNA concentrations tested and seemed to be more affected by the treatment time, increasing their concentration when increasing the treatment time.

In short, increasing the PTs resulted in an increase of the SKN, SCOD, proteins and carbohydrates. The increase of different organic and nitrogenous compounds is related to the increase in the hydrolysis rate (Wang et al. 2013). The maximum values of SCOD (25±3%) were obtained at an exposure time of 5 h independently of the FNA concentration applied, while the maximum values of SKN (53±3%.) were obtained at an exposure time of 5 h and 650 mg of N-NO_2^-/L. This demonstrates that FNA pre-treatment at low exposure times improve the hydrolysis step in PS. The reason could be that FNA and its derivatives such as nitric oxide (NO) or nitrous anhydride (N_2O_3) enhances EPS degradation (Bai et al., 2016; Dedon and Tannenbaum, 2004; Ma et al., 2015), solubilizing some of the particulate substrates. Also, it is important to highlight that the increase in SKN was not linked to an increase on ammonia concentration (Fig. 3D, 3E), indicating that the biomass was not able to hydrolyse the organic nitrogen. These results are in agreement with previous studies (Wang et al. 2013, 2014; Zahedi et al. 2016 and Ma et al. 2015) that also observed a positive effect of long FNA pre-treatment times (24h) on primary and secondary sludge solubilitzation.

3.4. Effect of FNA on methane production

In order to assess the potential inhibitory effect of the nitrite present in the FNA pre-treated biomass on the inoculum sludge used in the BMP tests, four different tests were conducted using only inoculum sludge and adding nitrite to reach three different nitrite concentrations (63, 90 and 117 mg N-NO_2^-/L mimicking the nitrite levels expected in the BMP tests were FNA pre-treated sludge was used as substrate). The results obtained are shown in Figure 4. Similar methane production was observed in all the blanks indicating that no inhibition occurred at the nitrite concentrations used in this study. No inhibition by similar concentrations of nitrite on the inoculum sludge was also reported in other studies (Wang et al., 2013, 2014; and Zahedi et al., 2016). The test without nitrite addition (blank I) was chosen as the blank to calculate the methane production from the pre-treated sludge.

Figure 5 shows the cumulative SMP in all the tests conducted. All results presented are already corrected subtracting the methane production obtained in the blank (inoculum). At the lowest pre-treatment time tested (1 h) the major improvement on SMP was observed for Test 3 and Test 4 corresponding to the highest FNA tested, increasing its SMP 7 and 16% respectively as compared with the untreated PS or as compared with Test 1 (PS subject to mixing but without FNA). Interestingly, when the pre-treatment times were increased to 2 and 5h the highest SMP was detected in Test 1, with mixing but without FNA, thus suggesting that applying a mild mixing in the PS for a period of 2h or longer was more effective than adding FNA to enhance methane production. The methane increase found in this test (between 14 and 17%) was similar to the one reported by Peces et al. (2016). They investigated PS pre-fermentation and detected a methane production potential increase of 14% when pretreating PS during 72 h under semi-aerobic conditions and concluded that this pre-treatment was suitable for this type of sludge.

Tests 2 and 3 also resulted in higher SMP production as compared to the untreated PS but these increments were lower (between 2 and 11%) than the one obtained in Test 1. Despite this, it is important to highlight that the highest SMP improvement (14-17%) was obtained for the PS pre-treated with 650 mg N-NO_2^-/L during 1 hour or a mild mixing in the PS for a period of 2h or longer.

These results differ from those obtained by Zhang et al. (2016) reporting a reduction on the methane production potential of 1–7% in those tests where FNA pre-treatment was applied. However, in their case a long pre-treatment time (24h) was used as compared with the pre-treatment times used in this study (1-5h). A recent study has also demonstrated that an increase on the FNA pre-treatment time in mixed sludge could result in a reduction on the methane reduction potential (Zahedi et al., 2016). Therefore, it is important to use low pre-treatment times when treating PS with FNA.

3.5.Economic viability of FNA pretreatment on PS

FNA pre-treatment on PS is only effective in increasing SMP at high concentrations of FNA (650 mg N-NO_2 /L) and low PTs (1h). The increase of SMP under these conditions (16%) is comparable to applying a mild mixing in the PS for a period of 2h (14%) or longer (17% at 5h). Therefore, the PS pre-treatment with FNA would only be economically feasible when a source of nitrite is available on site, for example in the case of those WWTP where a partial nitritation reactor is installed to treat reject wastewater (Law et al., 2015; Wang et al., 2014). In WWTP in which FNA is not available on site and nitrite needs to be purchased to produce the FNA, this pre-treatment for PS would not be economically viable, due to the extra costs of the chemical reagents.

A theoretical scale-up of this technology was conducted for a full-scale WWTP considering a PS influent flow rate in the digester of 77 m^3/d with a VS content of 33 g/Kg and gas production volume of 1400 m^3/d (being 65% of methane). The FNA pre-treatment cost would be around 78 €/d exposing the sludge at the best conditions found in this study (650 mg N-NO_2/ L) (http://www.alibaba.com/; Law et al., 2015), while the cost of only mixing the sludge at 5 h would be around 5 €/d (Mixing energy in the reactor= 0.13 kwh/m^3; Power Price= 0.0958€/kwh).

Regarding to the net gains, it depends on the biogas use and the country (Muñoz et al., 2015). Injection of biogas in a natural gas grid and use as a vehicle fuel are the best options.

More studies about FNA-pre-treatment at low exposure times in WAS are necessary, since its effectivity at low pre-treatment times has only been tested in mixed and primary sludge. This aspect is important, since low pre-treatment times are preferred for real application, reducing the volume of the pretreatment tank needed.

Conclusion

The effectiveness of the FNA pre-treatment applied to PS was assessed in this study. The following conclusions have been obtained:

- pH control is not necessary during FNA pre-treatment on PS if the pH of this sludge is already around 5.5.
- FNA improves the biodegradability of PS (SCOD, SKN and damaged cells).
- FNA treatment for PS, at exposure times lower than 5h can increase the MP to a maximum of 16% (at PTs of 1h and 650 mg N-NO_2/L).
- Mild agitations at 25ºC of the PS also improve MP (around 14-17%).
- Mild agitation is preferred to FNA pre-treatment for primary sludge due to the extra costs associated with the acquisition of nitrite when this is not available in the plant.

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Figure captions

Figure 1. pH evolution (A) and FNA evolution (B) from the PS with and without nitrite pre-treatment along the experiment.

Figure 2. Fractions of viable cells in the PS exposed to different FNA concentrations during different times.

Figure 3. Biomass specific production of SCOD (A), carbohydrates (B), proteins (C), SKN (D) and NH_4^+ (E) after FNA pre-treatment at different times. Error bars represent standard errors of triplicate samples.

Figure 4. Cumulative methane production from the inoculum exposed to different nitrite concentrations.

Figure 5. Cumulative SMP from the FNA pre-treated PS at different pre-treatment times: A- 1 h; B- 2 h; and C- 5 h. Error bars represent the standard error of triplicate samples.

Table 1. Experimental conditions applied during the PS pre-treatment with the FNA concentration varied by adjusting the nitrite concentration and the pH level.

25ºC	**Control***	**Test 1**	**Test 2**	**Test 3**	**Test 4**
FNA** (mg N-HNO_2/L)	0	0	3.9-1.2	5.6-1.7	7.3-2.6
Nitrite (mg N-NO_2^-/L)	0	0	350	500	650
pH***	5.3	5.3-5.3	5.3-5.8	5.3-5.8	5.3-5.8
Mild mixing	No	Yes	Yes	Yes	Yes
Time of exposure (h)					
1		X	X	X	X
2		X	X	X	X
5		X	X	X	X

*Control sludge (without any treatment (not agitation and neither FNA)

**The FNA range represents the initial and final FNA value found during the pre-treatment

***The pH range represents the initial and final pH value found during the pre-treatment

Figure 1.

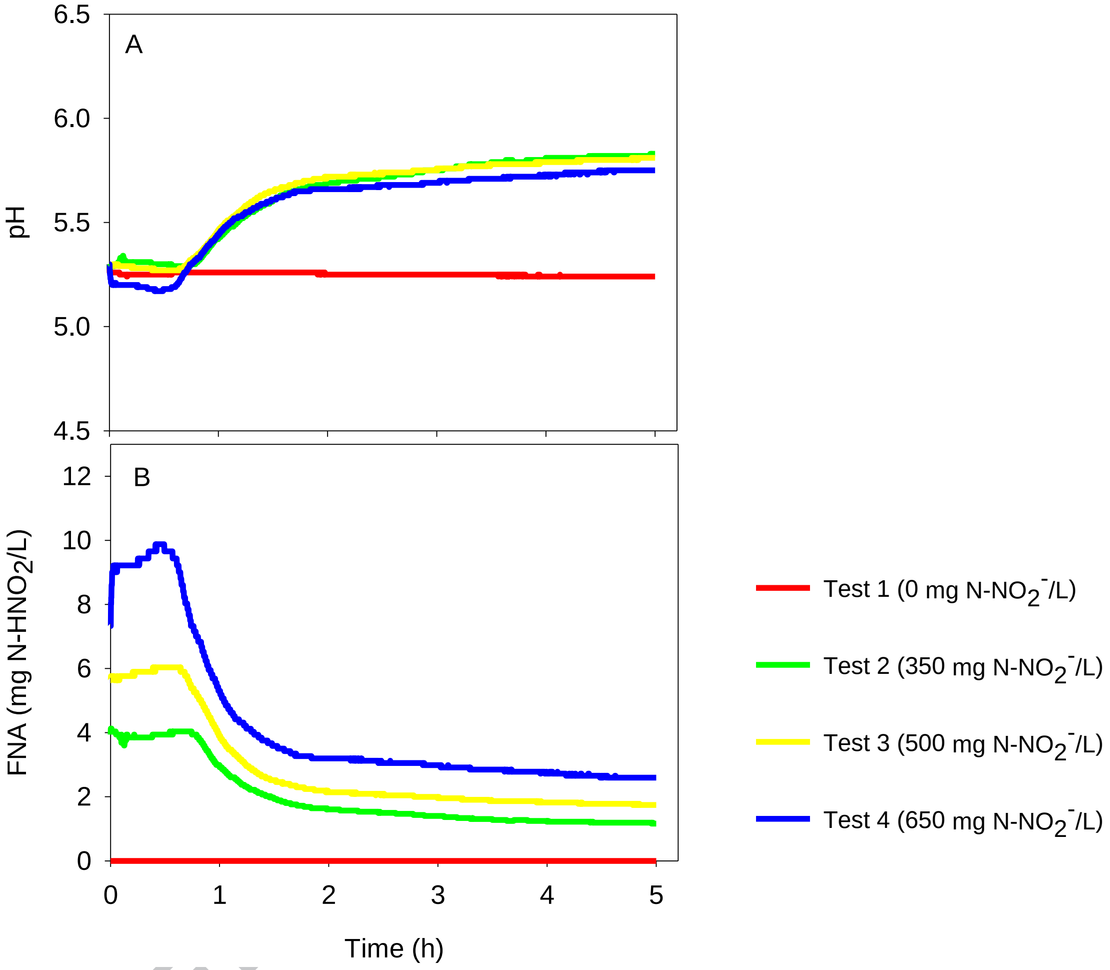

A
pH
6.5
6.0
5.5
5.0
4.5
B
FNA (mg N-HNO2/L)
12
10
8
6
4
2
0
0
1
2
3
4
5
Time (h)
Test 1 (0 mg N-NO2-/L)
Test 2 (350 mg N-NO2-/L)
Test 3 (500 mg N-NO2-/L)
Test 4 (650 mg N-NO2-/L)

Figure 2.

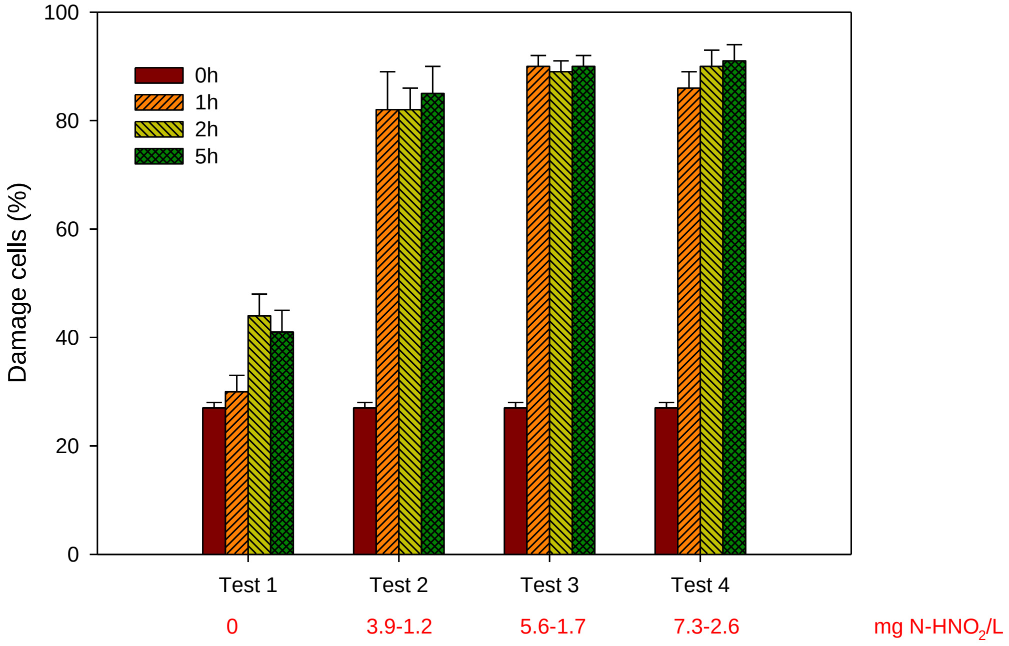

100
80
60
40
20
0
Damage cells (%)
0h
1h
2h
5h
Test 1
Test 2
Test 3
Test 4
0
3.9-1.2
5.6-1.7
7.3-2.6
mg N-HNO$_2$/L

Figure 3

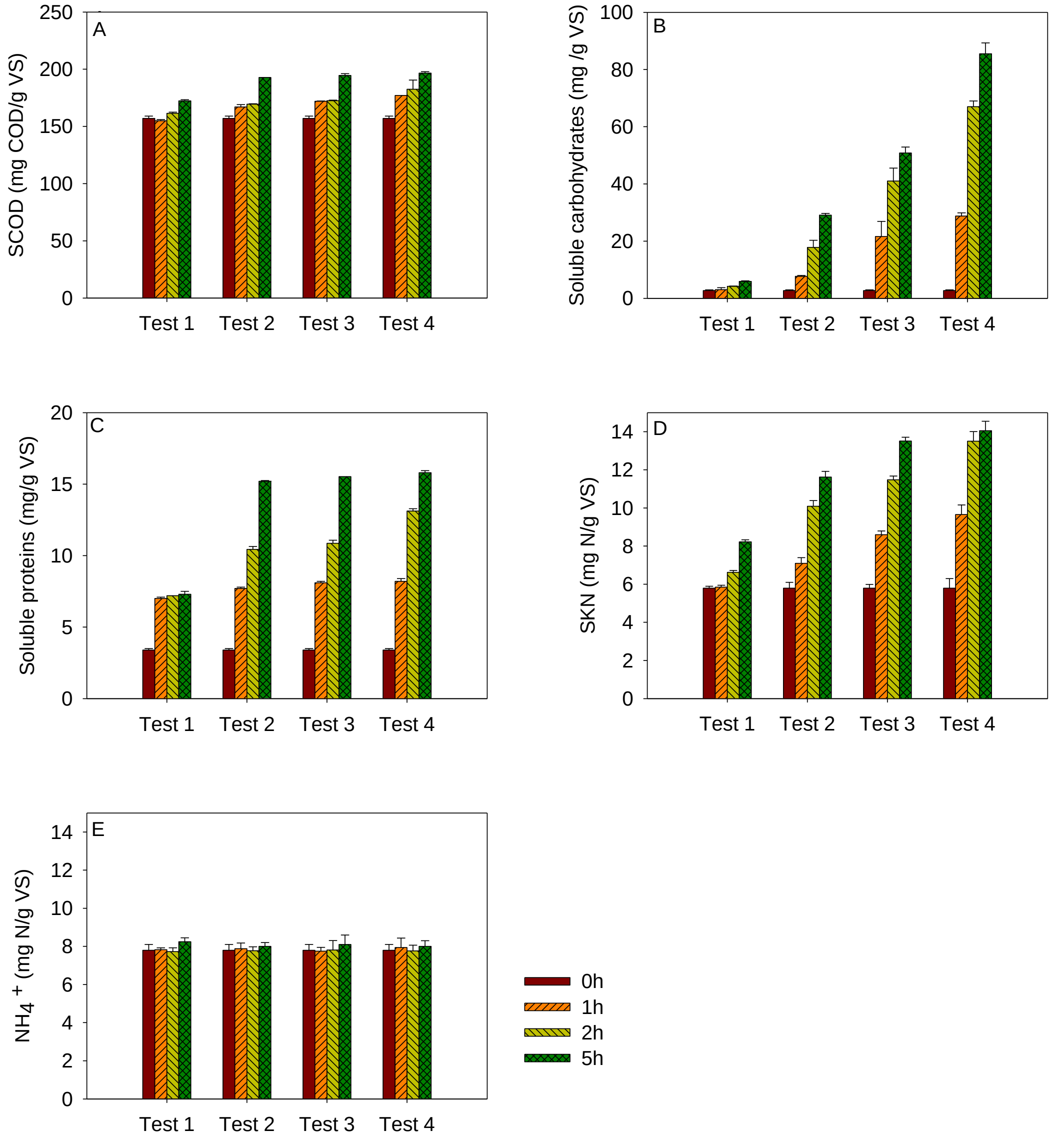

A
SCOD (mg COD/g VS)
0
50
100
150
200
250
B
Soluble carbohydrates (mg /g VS)
0
20
40
60
80
100
C
Soluble proteins (mg/g VS)
0
5
10
15
20
D
SKN (mg N/g VS)
0
2
4
6
8
10
12
14
E
NH_4^+ (mg N/g VS)
0
2
4
6
8
10
12
14
Test 1
Test 2
Test 3
Test 4
0h
1h
2h
5h

Figure 4.

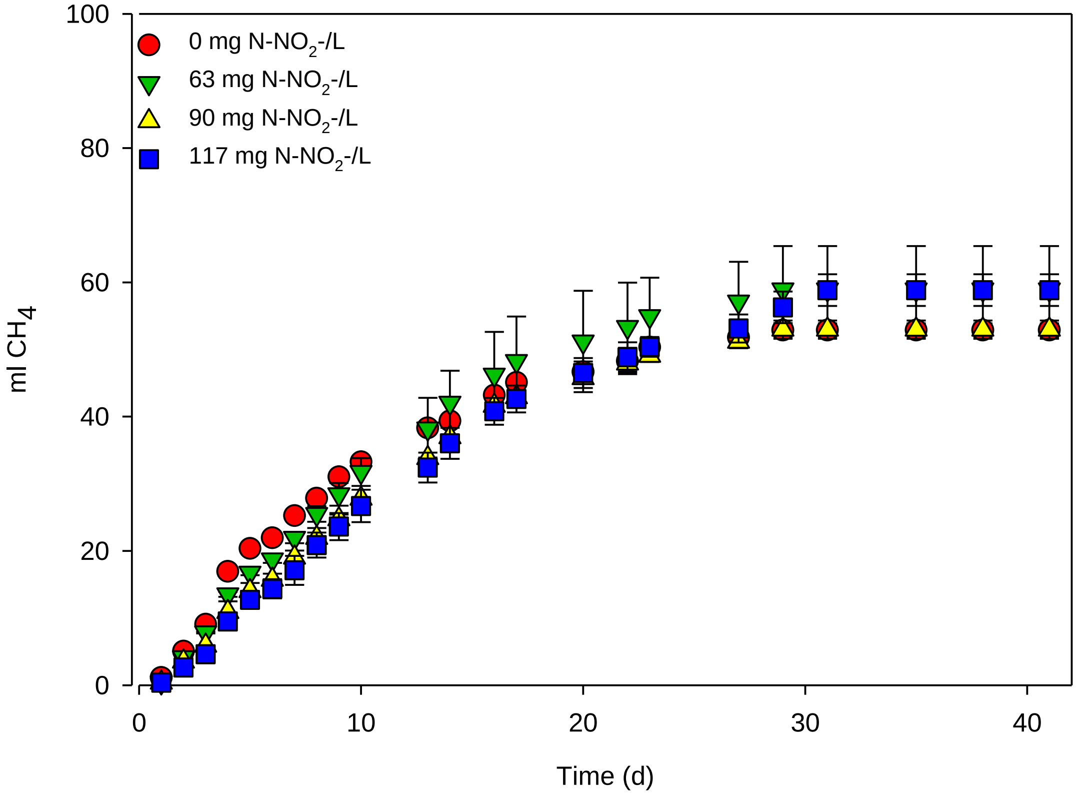
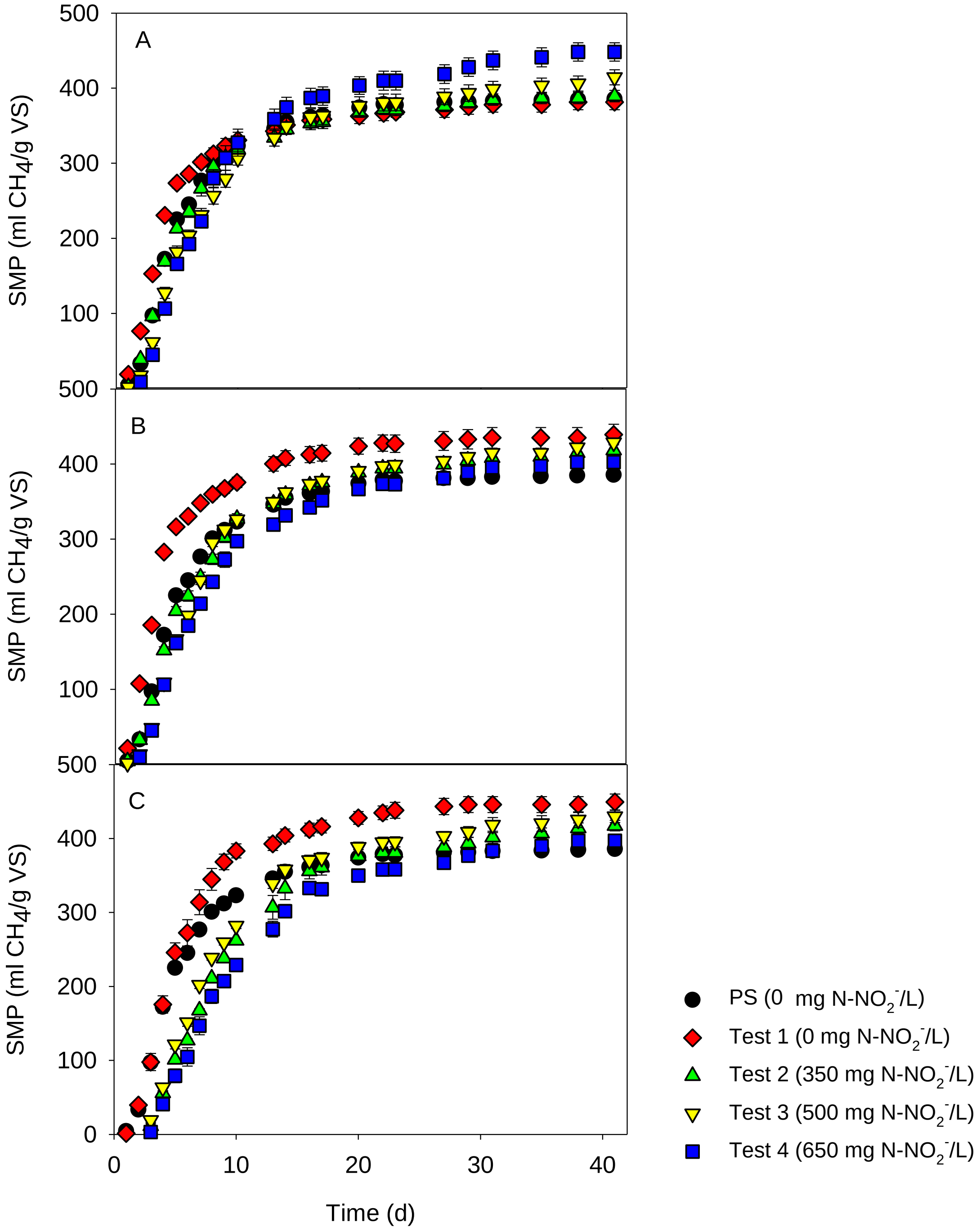

100
80
60
40
20
0
ml CH_4
0
10
20
30
40
Time (d)
0 mg $N\text{-}NO_2$-/L
63 mg $N\text{-}NO_2$-/L
90 mg $N\text{-}NO_2$-/L
117 mg $N\text{-}NO_2$-/L

Figure 5.


A
B
C
SMP (ml CH4/g VS)
500
400
300
200
100
0
0
10
20
30
40
Time (d)
PS (0 mg N-NO2-/L)
Test 1 (0 mg N-NO2-/L)
Test 2 (350 mg N-NO2-/L)
Test 3 (500 mg N-NO2-/L)
Test 4 (650 mg N-NO2-/L)

Highlights

- pH control is not necessary during FNA pre-treatment on primary sludge (PS)
- FNA increases the solubility and reduces cell viability (<20%)
- At 1h, FNA (with 650 mg N-NO_2^-) provided the highest methane production (MP)
- Similar enhancement on MP were obtained while subjecting the PS at mild at 2-5 h
- FNA is not necessary to increase MP; mild agitations also improve MP (around 14-17%)